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Management history determines gene flow in a prominent invader

Waafeka Vardien, David M. Richardson, Llewellyn C. Foxcroft, John R. U. Wilson and Johannes J. Le Roux

W. Vardien, D. M. Richardson, L. C. Foxcroft, J. R. U. Wilson and J. J. Le Roux (jacoleroux01@gmail.com), Centre for Invasion Biology, Dept of Botany and Zoology, Stellenbosch Univ., Matieland 7602, South Africa. LCF also at: Conservation Services, South African National Parks, Skukuza 1350, South Africa. JRUW also at: South African National Biodiversity Inst., Kirstenbosch National Botanical Gardens, Claremont 7735, South Africa.

Invasive plants pose substantial threats to protected areas globally. Although management can limit impacts, spread and reinvasion from neighbouring areas into protected areas are a major and an on-going problem for land managers. However, identifying the main sources of propagules and the dimensions of invasion pathways is challenging. This study used population genetic markers [inter simple sequence repeats (ISSRs) and amplified fragment length polymorphisms (AFLPs)] to infer the source(s) of re-colonization and dispersal patterns for a typical invader of riparian and terrestrial habitats (*Lantana camara*) along the Sabie-Sand catchment, one of the most important river systems flowing into and across South Africa's flagship protected area, the Kruger National Park (KNP). Results indicate that populations located along the lower reaches of the Sabie and Sand tributaries harboured substantially higher genetic diversity than those in the upper Sabie catchment. Bayesian assignments indicated that the upper Sabie tributary contributed far fewer propagules than the Sand tributary to the lower Sabie River. Current invasion patterns are due to a combination of a major flood event in 2000 and differences in the degree to which the upstream reaches were managed after the flooding. The major flood of 2000 effectively cleared lantana from the riparian areas. However, whereas on-going management efforts against riparian species in the KNP have been effective, rendering the upper Sabie relatively clear of lantana, only a small part of the Sand tributary falls under jurisdiction of the KNP and has received consistent management attention. The reinvasion of the lower Sabie in the KNP was therefore almost entirely by propagules from the Sand tributary. The study highlights the important role that molecular tools can play in determining dispersal dynamics and directing invasive species management. For invasive plant species that invade both riparian habitats and landscapes away from rivers in protected areas, such as lantana, management must focus on all major sources of propagules to limit reinvasion.

Some invasive alien species cause substantial ecological damage and pose major threats to biodiversity (Gurevitch and Padilla 2004), to the extent that biological invasions are an important driver of global environmental change (Butchart et al. 2010). Protected areas form one of the most important opportunities for conserving biodiversity and ecosystem services globally (Gaston et al. 2008), and invasive species directly threaten the ability of these areas to meet their mandate. Invasive species affect ecosystem services (Vilà et al. 2010), disrupt fire regimes (D'Antonio and Vitousek 1992, Rossiter-Rachor et al. 2009) and nutrient cycling (Ehrenfeld 2003), and have direct impacts on native species (Vonshak et al. 2010). As key focus areas for conservation, protected areas can, and should, be at the forefront of systematic management of invasive species. However, limited resources often result in insufficient attention being given to objective prioritization and the integration of all available information to ensure effective management.

Eradication is feasible for some species, but for most widespread species complete removal is untenable (Rejmánek and Pitcairn 2002). For widespread invaders, efficient management requires a thorough understanding of the many factors that contribute to persistence, proliferation and spread, such as life-history traits, seed bank dynamics, dispersal vectors and the role of propagule pressure (Lockwood et al. 2007). Different mechanisms affect invasions at different stages and these interact in complex ways with features of the receiving environment. For example, dispersal vectors (birds, roads, rivers, humans and animals) are particularly important as they can exacerbate the spread of invasive species from outside sources into protected areas (Naiman and Décamps 1997, Pauchard and Alaback 2004, Dovrat et al. 2012).

Most protected areas are embedded in a landscape of unprotected land and can be considered 'islands' in a sea of different land uses, onto which urban development is rapidly encroaching (McDonald et al. 2008). Although alien species are sometimes introduced directly into protected areas, the surrounding unprotected areas often represent a significant potential source and multiple pathways of

introduction and opportunities for reinvasion (Foxcroft et al. 2011). The management of invasive plant species in protected areas thus needs to consider the configuration of the park in relation to external propagule sources. For example, at large spatial scales knowledge of the primary sources of invasions helps to determine the risks of re-invasions and can be used to prioritize areas for management outside the protected area. Similarly, invasive species that share key life-history traits, e.g. dispersal and reproduction, will likely require similar management interventions.

A specific example of the problems outlined above is the threat posed by the spread of invasive species along watercourses. If an entire watershed is not included within the boundaries of the protected area, rivers can be significant conduits for the movement of plant propagules into protected areas (Naiman and Décamps 1997, van Wilgen et al. 2007, Jarošík et al. 2011). For instance, in South Africa's flagship protected area, the Kruger National Park (KNP), some of the worst invasive plants have been introduced, and are spreading along major rivers (Foxcroft et al. 2008a). Although work has been done on prioritising control projects (Forsyth et al. 2012 and references therein), insufficient attention has been given to understanding where species spread from and how this can be incorporated into strategic management plans.

We investigated the dispersal dynamics for a typical 'riparian-landscape' invasive plant (species that invade both riparian habitats and terrestrial habitats away from rivers; Rouget et al. 2004), using the invasive plant species complex, *Lantana camara* L. (sensu lato) (hereafter referred to as *L. camara*) in the KNP as a model system. *Lantana camara*, a weed of global concern (Day et al. 2003), was first observed in the KNP in the 1940s (Vardien et al. 2012). In the 1950s it was planted extensively as an ornamental in staff villages and rest camps and soon became naturalized along rivers. *Lantana camara* is widespread along the entire course of the Sabie River, and some areas are covered by extensive, impenetrable thickets, especially the upper catchment (Vardien et al. 2012).

The management of most alien plant species in and adjacent to the KNP is primarily funded by the South African government's Working for Water (WfW) Programme, the national body responsible for managing invasive plants. To date, WfW has spent approximately 180 million ZAR (7% of its total budget) on controlling *L. camara* invasions in South Africa (van Wilgen et al. 2012). However, the programme's success against *L. camara* invasions has been limited as cleared sites are often quickly reinvaded (Euston-Brown et al. 2007).

Lantana camara reproduces both sexually and vegetatively, and is associated with multiple dispersal vectors (Day et al. 2003). If the dispersal pathway(s) and source(s) were determined, management intervention could be applied strategically to minimise propagule pressure and reduce re-colonization and spread into the KNP, not only of *L. camara*, but also co-occurring invaders with similar dispersal strategies. Determining dispersal patterns at the landscape-scale may also help to identify the most appropriate spatial scale for planning management efforts – e.g. whether to focus attention on individual tributaries or to work towards integrated plans for entire catchments. If an invasive species readily re-colonizes cleared areas, then management arguably needs to simultaneously target the entire network of connected populations (Hampton et al. 2004). Alternatively, understanding how populations are connected can be used to plan the order in which populations need to be controlled (Chades et al. 2011).

Determining dispersal patterns is unfortunately not straightforward for sessile plants and therefore patterns of gene flow are often used as a proxy for dispersal (Ouborg et al. 1999, Le Roux and Wieczorek 2009). This study aims to identify the main contributing source(s) of propagules of *L. camara* invasions into KNP by analysing the amount and distribution of genetic diversity within and among populations along the Sabie-Sand River catchment.

A riparian-landscape invader can spread in many different ways (especially in a region with a complex structure of human influence), but based on the history of *L. camara*'s distribution in the KNP and its management, we consider three main scenarios, each associated with specific predicted genetic signatures and management implications (Fig. 1). *Lantana camara* fruits are eaten and dispersed by birds. If dispersal by birds was most important, we would predict that populations could be admixed or randomly distributed throughout the landscape (no barriers to dispersal) resulting in similar gene diversity across populations and an absence of isolation by distance (Fig. 1, scenario (a)). This pattern should not be vastly influenced by differential management among tributaries and for reinvasions to be limited; a buffer zone would need to be kept clear right around the park. Alternatively, if seed dispersal (in water) along rivers is more important, the headwaters outside KNP would serve as the main source areas for reinvasions. Moreover, because there are two tributaries in the upper catchment, there may be equal or differential contributions of propagules to the lower tributary (Fig. 1, scenarios (b) and (c)). As such, management would have to focus on keeping the tributaries clear to limit reinvasions. Such dispersal would also give rise to a particular genetic signal. If reinvasion was from both tributaries, then there would be structured genetic diversity between these two tributaries, novel genetic entities in the lower Sabie and isolation by distance. On the other hand, if one tributary was the main source for reinvasion, then we would expect structured genetic diversity between the upper Sabie and Sand tributary, and less isolation by distance between the dominating upper tributary and lower Sabie. Clearly in this third case, more invasive alien plant clearing is required in one particular catchment.

We believe that the elucidation of the case study of *L. camara* in the Kruger National Park could serve as a protocol for the integration of insights from the application of molecular methods and other methods in formulating more robust strategies for managing invasive alien plants.

Material and methods

Study site

The study was conducted along the Sabie and Sand Rivers in the KNP (Fig. 2). Both rivers originate in the Mpumalanga highlands, with the Sand merging with the upper Sabie just

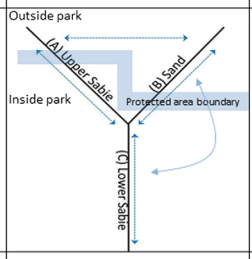
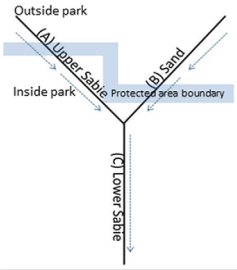
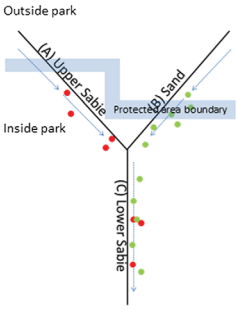
Dispersal mechanism	Schematic	Predicted genetic signatures	Management implications
(a) Long distance (bidirectional) bird dispersal between stretches of river (A) – (B) – (C).		• Genetic diversity randomly distributed or similar amongst populations • No isolation by distance	• Clearing of catchments only insufficient • Establish buffer zone larger than dispersal distances of vectors around protected area
(b) Dispersal downstream along the river from (A) to (C) and from (B) to (C). Both (A) and (B) contribute equally to (C).		• Accumulation of genetic diversity/ novel entities down-stream (C) • Genetic diversity structured • Isolation by distance present within tributaries	• Clearing of entire catchment needed for effective control
(c) Dispersal downstream along the river from (A) to (C) and from (B) to (C). One tributary (A) or (B) dominating.		• Accumulation of genetic diversity and novel genetic entities down-stream (C) • Genetic diversity structured • If (A) dominates, less isolation by distance is expected between (A) and (C), (A) is likely the source of invasions and vice versa	• Refocusing clearing efforts on dominating tributary, simultaneously clear all sources contributing propagules

Figure 1. Spread scenarios for *Lantana camara* populations along the Sabie-Sand catchment in and around Kruger National Park, their expected genetic signatures and management implications: (a) frequent long-distance dispersal across the landscape, e.g. due to frugivorous birds, (b) dispersal primarily downstream from both catchments, and (c) dispersal from one catchment predominates, indicating a primary source of propagules.

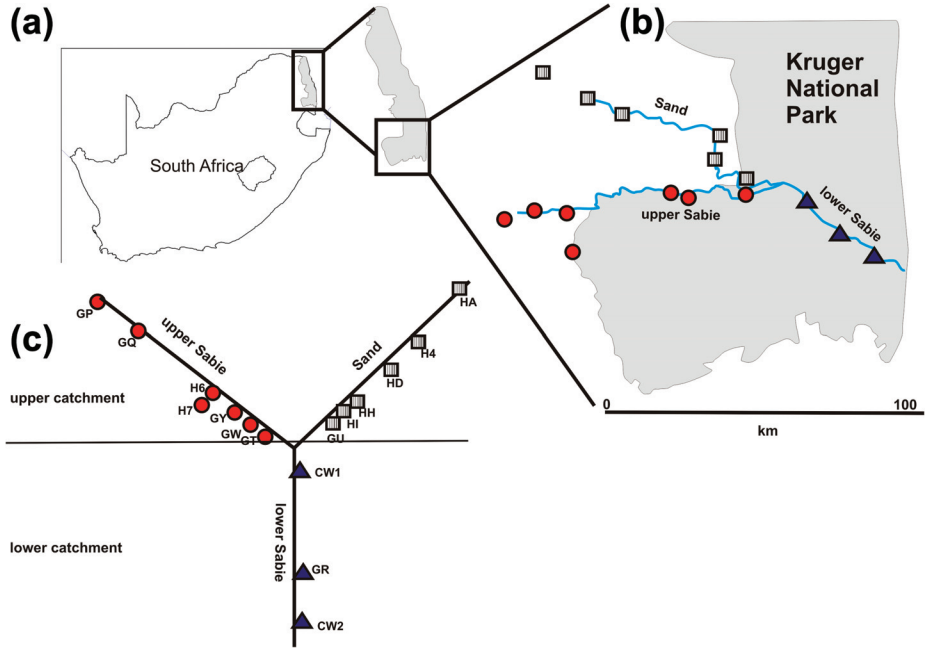


Figure 2. Study site and sampling localities: (a) Kruger National Park; (b) sampling sites along the Sabie-Sand catchment; and (c) a schematic diagram showing the landscape position of the populations sampled.

inside the KNP, from where the lower Sabie continues eastwards until its confluence with the Incomati River in Mozambique. The Sabie-Sand River catchment covers an area of approximately 7096 km^2 (Foxcroft et al. 2008b).

Population sampling and DNA extraction

Sixteen populations (groups of 12 or more individuals separated from another group by at least two kilometres) were sampled in the Sabie-Sand catchment (Table 1, Fig. 2). Due to active management within KNP, fewer populations remained to be sampled along the lower Sabie as compared to the upper Sabie and Sand regions, despite extensive search efforts all along the river. For each population, leaf material from 12 to 29 individual plants was collected, dehydrated, and kept on silica gel until processing.

Total genomic DNA was extracted from leaf material, following the cetyltrimethyl ammonium bromide (CTAB) method, described by Doyle and Doyle (1990). DNA quality was determined on a 1% agarose gel, and quantity measured using a spectrophotometer (NanoDrop ND1000, Thermo Scientific, Wilmington, DE, USA). In order to maximize reproducibility and comparability between samples during downstream processing, all DNA extractions were diluted to 20 ng/µl (for ISSRs) or 200 ng/µl (for AFLPs).

ISSR amplification

Fragments from four fluorescently labelled inter simple sequence repeats (ISSRs) primers $(GTC)_4RC$; $(CA)_6GA$; $(GA)_8C$ and $(GAC)_4RC$ were amplified. Amplification was carried out in 10 µl reaction volumes containing 1 µl of diluted genomic DNA, 0.8 µl of 10 µM primer, 0.2 µl of 20 mM dNTP mix (Southern Cross Biotechnologies, Cape Town, South Africa), 1 µl of 10× buffer, 0.6 µl of mM $MgCl_2$, 0.2 µl 5 u/µl Super-Therm Taq polymerase (Southern Cross Biotechnologies, Cape Town, South Africa) and 0.2 µl of 10 mg/ml BSA. PCR cycles were performed at initial denaturation of 95°C for 4 min, followed by 35 cycles at denaturation at 94°C for 40 s, appropriate annealing [primers $(GTC)_4RC$ and $(CA)_6GA$ at 45°C; $(GA)_8C$ at 49°C; and $(GAC)_4RC$ at 46°C] for 45 s, elongation at 72°C for 60 s; and final extension at 72°C for 10 min. Amplification success was confirmed by running and visualising PCR products on agarose gels of varying concentration [3% for $(GTC)_4RC$, 3.5% for $(CA)_6GA$ and $(GAC)_4RC$, and 1.5% for $(GA)_8C$] in 0.5× TBE buffer, at a

Table 1. Geographic location and tributary placement of sampled populations of *Lantana camara* (Fig. 1) with estimates of genetic diversity at 74 ISSR (top value) and 62 AFLP (bottom value) loci: effective number of alleles (Ne), Nei's gene diversity (*h*), Shannon's information index (I), and average panmictic heterozygosity (hs).

Population ID	n	Section of catchment	Latitude	Longitude	Ne	*h*	I	hs
CW1	22	lower Sabie	−24.995	31.767	1.500	0.243	0.432	0.325
					1.439	0.214	0.321	0.302
CW2	19	lower Sabie	−25.155	31.940	1.595	0.305	0.462	0.390
					1.498	0.293	0.437	0.376
GR	22	lower Sabie	−25.067	31.843	1.405	0.231	0.374	0.299
					1.387	0.216	0.345	0.276
HI	18	Sand	−24.873	31.545	1.113	0.051	0.083	0.123
					1.103	0.046	0.071	0.122
HH	24	Sand	−24.823	31.549	1.128	0.118	0.142	0.124
					1.112	0.105	0.134	0.112
HA	14	Sand	−24.633	31.041	1.194	0.119	0.140	0.122
					1.187	0.109	0.121	0.117
GU	19	Sand	−24.957	31.606	1.535	0.292	0.435	0.305
					1.501	0.285	0.404	0.299
HD	14	Sand	−24.779	31.294	1.324	0.163	0.255	0.134
					1.302	0.142	0.231	0.128
H4	21	Sand	−24.727	31.230	1.168	0.109	0.203	0.104
					1.162	0.101	0.186	0.098
GT	15	upper Sabie	−24.993	31.601	1.372	0.129	0.338	0.246
					1.250	0.106	0.238	0.234
H6	12	upper Sabie	−25.052	31.130	1.079	0.038	0.047	0.124
					1.002	0.023	0.039	0.112
H7	19	upper Sabie	−25.141	31.167	1.120	0.096	0.096	0.124
					1.070	0.089	0.092	0.111
GP	11	upper Sabie	−25.056	30.950	1.167	0.082	0.122	0.142
					1.126	0.074	0.103	0.131
GQ	28	upper Sabie	−25.028	31.025	1.164	0.101	0.124	0.117
					1.132	0.099	0.121	0.111
GY	22	upper Sabie	−24.988	31.435	1.095	0.127	0.077	0.107
					1.001	0.108	0.062	0.102
GW	29	upper Sabie	−24.990	31.457	1.211	0.050	0.206	0.108
					1.209	0.041	0.199	0.106

constant voltage of 110 V for 40 min. PCR-amplified products were purified using the NucleoFast 96 PCR membrane (Macherey-Nagel, Germany), and genotyped using an ABI PRISM 377XL DNA sequencer (PE Applied Biosystems, Foster City, CA, USA) and fragments sized relative to a molecular size marker (LIZ 500, PE Applied Biosystems, Foster City, CA, USA). For each individual, each locus (size fragment) was scored as present or absent ('1' = locus present, '0' = locus absent) using GeneMarker ver. 1.97 (SoftGenetics, LLC, CA, USA). Repeatability of both ISSR banding patterns was assessed by repeating PCR and genotyping as described above for a subset of samples.

AFLP amplification

Amplified fragment length polymorphism (AFLP) amplification was modified based on Vos et al. (1995) and Vuylsteke et al. (2007). DNA templates were prepared in a 20 µl reaction volume containing 1 µl of 200 ng/µl genomic DNA, 0.5 µl of 1× EcoRI (Fermentas, supplied by Inqaba Biotech, Pretoria, South Africa), 4 µl of 10× Buffer Tango (Fermentas, supplied by Inqaba Biotech, Pretoria, South Africa), and 14.5 µl distilled water digested at 37°C for 1 h. Thereafter 0.5 µl of Tru1I (Fermentas, supplied by Inqaba Biotech, Pretoria, South Africa), 2 µl of 10× Buffer Tango (Fermentas, supplied by Inqaba Biotech, Pretoria, South Africa), and 7.5 µl distilled water was added to the reaction mix, and further digested for 1 h at 65°C.

Before ligation, double-stranded adaptor pairs were constructed from complementary single-stranded oligonucleotides (EcoRI: 5′-CTCGTAGACTGCGTACC-3′ and 3′-CATCTAGACGCATGGTTAA-5′; MseI: 5′-GACGA TGAGTCCTGAG-3′ and 3′-CTACTCAGGAC TCAT-5′). The EcoRI adaptor pair was prepared by combining 5 µl of 100 µM of each oligonucleotide with 90 µl of distilled water and the MseI adapter pair by combining 25 µl of 100 µM of each oligonucleotide, and then heating the reactions to 95°C for 10 min, followed by 5 min at 65°C, and cooling down to room temperature.

The adapters were then ligated to template DNA for 2 h at 22°C in a 10 µl reaction volume consisting of 1 µl of T4 DNA ligase (Fermentas, supplied by Inqaba Biotech, Pretoria, South Africa), 1 µl of 10× T4 DNA ligase buffer (Fermentas, supplied by Inqaba Biotech, Pretoria, South Africa), 1 µl of 5 µM EcoRI adapter, 1 µl of 50 µM MseI adapter and 6 µl of distilled water. Thereafter the reaction was diluted 5× for use as template DNA in a pre-selective amplification.

Pre-selective amplification was carried out in 15 µl reaction volumes containing 1.5 µl template DNA (as prepared above), 0.75 µl of 20 µM EcoRI primer (5′-GAC TGCGTACCAATTC-3′), 0.75 µl of 20 µM MseI primer (5′-GATGAGTCCTGAGTAA-3′), 7.5 µl of 2× Kapa Taq ReadyMix (Kapa Biosystems, supplied by Inqaba Biotech, Pretoria), and 4.5 µl distilled water. PCR cycles were performed as described in Vuylsteke et al. (2007) and products were diluted 5× for use in selective AFLP amplification.

Four AFLP primers were selected for amplification: EcoRI-ATG; EcoRI-CAT; EcoRI-AAT and MseI-CTT. Amplification was carried out in 20 µl reactions containing 5 µl DNA template (as prepared by pre-selective amplification above), 0.5 µl of 20 µM EcoRI primer, 1.5 µl of MseI primer, 10 µl of 2× Kapa Taq ReadyMix (Kapa Biosystems, supplied by Inqaba Biotech, Pretoria, South Africa), and 12 µl of distilled water. PCR cycles and amplification success was confirmed as described in Vuylsteke et al. (2007). Purification, genotyping, scoring and examining repeatability was performed as described for the ISSRs above.

Genetic diversity

ISSR and AFLP datasets were analysed separately, as well as in combination. Genetic diversity was estimated as the number of effective alleles (Ne), Nei's (1973) gene diversity (*h*) and Shannon's diversity index (I) (Shannon and Weaver 1949) using PopGene ver. 1.32 (Yeh et al. 1995). As estimates derived from PopGene 1.32 assume populations are in Hardy–Weinberg equilibrium (HWE), a Bayesian approximation, which is less sensitive to the assumptions of HWE, was also used (Holsinger et al. 2002), and the average panmictic heterozygosity (hs) was estimated using the f-free model in Hickory ver. 1.1 (Holsinger and Lewis 2003). We analysed the distribution of population genetic variation within and between populations using an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) as implemented in GenAlEx6 ver. 6.41 (Peakall and Smouse 2006) using ΦPT (analogous to Wright's Fixation index or FST) as the measure of genetic distance.

Genetic structure

The genetic structure of *L. camara* populations was assessed using two approaches and the combined AFLP/ISSR dataset. First, a principle components analysis (PCA) was performed to visualize genetic clustering between populations in a multi-dimensional space using GenAlEx6 ver. 6.41 (Peakall and Smouse 2006). Second, Bayesian assignment tests were performed to assign individual genotypes probabilistically to populations using STRUCTURE ver. 2.3.2 (Falush et al. 2007). Simulations were run with between one and ten populations or genetic clusters (K), using the option of taking population affiliation into account, and allowing admixture. Two runs of 100 000 iterations followed by a burn-in period of 100 000 for each K value were performed. In addition, LnProb values obtained from the STRUCTURE analysis were used to calculate ΔK (Evanno et al. 2005) to estimate the optimum number of genetic clusters.

To test for isolation by distance, Mantel tests with 1000 permutations implemented in IBDWS ver. 3.16 were used (Jensen et al. 2005) comparing two estimates of geographical distance: the Euclidian distance, and the distance along the river, to pairwise population genetic distances (ΦPT).

Results

Genetic diversity

All *L. camara* individuals included here were diploid (Supplementary material Appendix 1–3, Table A1, Fig. A1). A total of 136 (74 ISSR and 62 AFLP) loci were reliably scored for every individual. Re-analysis (i.e. re-amplification, re-genotyping and re-scoring) of 16% (ISSR) and 9% (AFLP) of the total sample sizes revealed high repeatability with an estimated error rate of 3.2% (ISSR) and 4.3% (AFLP) respectively. The effective number of alleles (Ne) ranged from 1.079 to 1.595 for ISSR loci and from 1.001 to 1.501 for AFLP loci (Table 1).

Nei's gene diversity (*h*) ranged from 0.038 to 0.305 (mean = 0.141) for ISSR loci and from 0.023 to 0.293 (mean = 0.128) for AFLPs. Shannon's information index (I) values ranged from 0.047 to 0.462 (mean = 0.221) for ISSRs and from 0.039 to 0.437 (mean = 0.194) for AFLPs. Average panmictic heterozygosity (hs) for ISSRs ranged from 0.104 to 0.390 (mean = 0.181) and for AFLPs from 0.098 to 0.376 (mean = 0.172) (Table 1). Genetic diversity indices were generally highest in populations of the lower Sabie, with the exception of population GU (Sand) and GT (upper Sabie) which were sampled near the Sabie-Sand confluence (Table 1). The majority of genetic variation resided within rather than between populations (Table 2).

Genetic structure

The PCA illustrated clear spatial structure. Populations from the Sand and lower Sabie formed a cluster distinct from those along the upper Sabie (Fig. 3). The first two axes explained 81.5% of the cumulative variation. The structure analysis and PCA were in agreement, showing that populations sampled in this study can be assigned to two distinct clusters (Fig. 4).

The first corresponds to the upper Sabie and the second, to the Sand and lower Sabie. Moreover, little genotypic information (few alleles) is shared between the upper Sabie and the Sand, and the upper Sabie has contributed substantially less genotypic information to the lower Sabie than the Sand.

These results appear to be consistent with the isolation by distance analysis. When all populations were considered using the ISSR data (Fig. 5a, d), there was a pattern of isolation by distance. Populations further away tended to be less genetically related. When the analysis was split the pattern became clearer. There is no isolation by distance between the Sand and the lower Sabie (Fig. 5c, f) but strong isolation by distance between upper and lower Sabie (Fig. 5b, e), and this relationship is more statistically significant if distance along river was used as opposed to Euclidean distance ($p < 0.001$ vs $p < 0.024$). Analysis of the AFLP loci showed similar results (data not shown).

Table 2. Results of AMOVA comparing the distribution of variation between and within populations of *Lantana camara* along the Sabie-Sand catchment.

Source of variation	% variation	ΦPT
ISSR		
Among populations	46	0.463*
Within populations	54	
AFLP		
Among populations	49	0.482*
Within populations	51	

*$p < 0.01$.

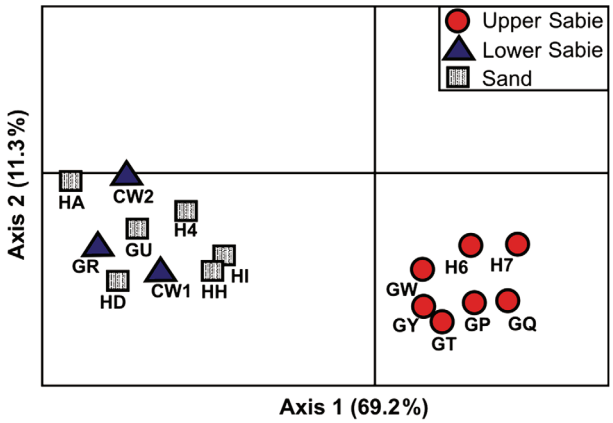


Figure 3. Plot of the first two axes of a PCA showing genetic differentiation based on pairwise ΦPT values for *Lantana camara* derived from ISSR and AFLP loci. Sand and lower Sabie populations cluster together, and upper Sabie populations cluster separately.

Discussion

Here we show that the effective management of a landscape-riparian invader (e.g. *L. camara* in the KNP) can be hampered by the continuous introduction of propagules from riparian sources outside the boundaries of the protected area. Specifically, the upper Sabie and Sand tributaries differ substantially in their relative contribution of propagules to the lower Sabie, with the Sand contributing substantially more than the upper Sabie. The genetic signature identified here best categorizes a scenario of continuous downstream river-driven dispersal (Honnay et al. 2010) dominated by one tributary (Fig. 1c), the Sand. Our results emphasize the importance of understanding the impacts and contributions of different landscape features, processes, and scales in shaping the invasion pathways of alien species into protected areas. Management efforts often target numerous species at particular sites; therefore assessment of propagule movement for a single taxon potentially provides a tractable way of gaining information on the overall management efforts in the area.

We consider numerous scenarios to account for these marked differences in propagule contributions to the lower Sabie tributary: differences in river hydrology and morphology, differences in the level of invasion within rivers, and differences in invasion histories of the tributaries, e.g. independent introductions or differences in residence times.

It is unlikely that the patterns observed are due to differences between rivers in flow rates, rainfall seasonality, or elevation. While the Sabie tributary is larger than the Sand, they are fairly similar in terms of the aforementioned

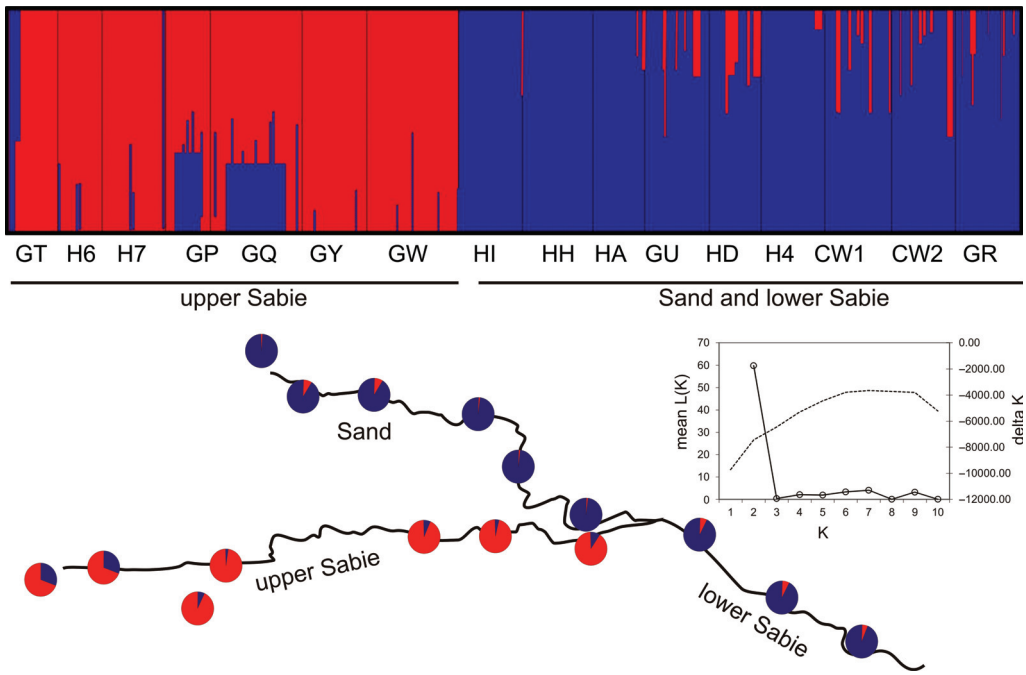


Figure 4. Population structure based on ISSR and AFLP loci, inferred from Bayesian assignment tests, showing that *Lantana camara* populations in KNP's Sabie-Sand catchment consists of two genetic clusters (identified based on the ΔK method by Evanno et al. (2005)). Vertical bars show each individual divided into coloured sections that represent the proportional membership of its genome to a particular K cluster. Pie charts are representative of the overall genome assignment of a population to a particular genetic cluster.

features. The Sand is 60 km long whereas the upper Sabie is 130 km long; both originate at similar elevations (Sabie: 2000 m, Sand: 1600 m); they fall within the same ecological region; and receive similar amounts of annual rainfall (State of Rivers Report 2001, Moolman et al. 2002). Consequently they probably do not differ substantially in terms of

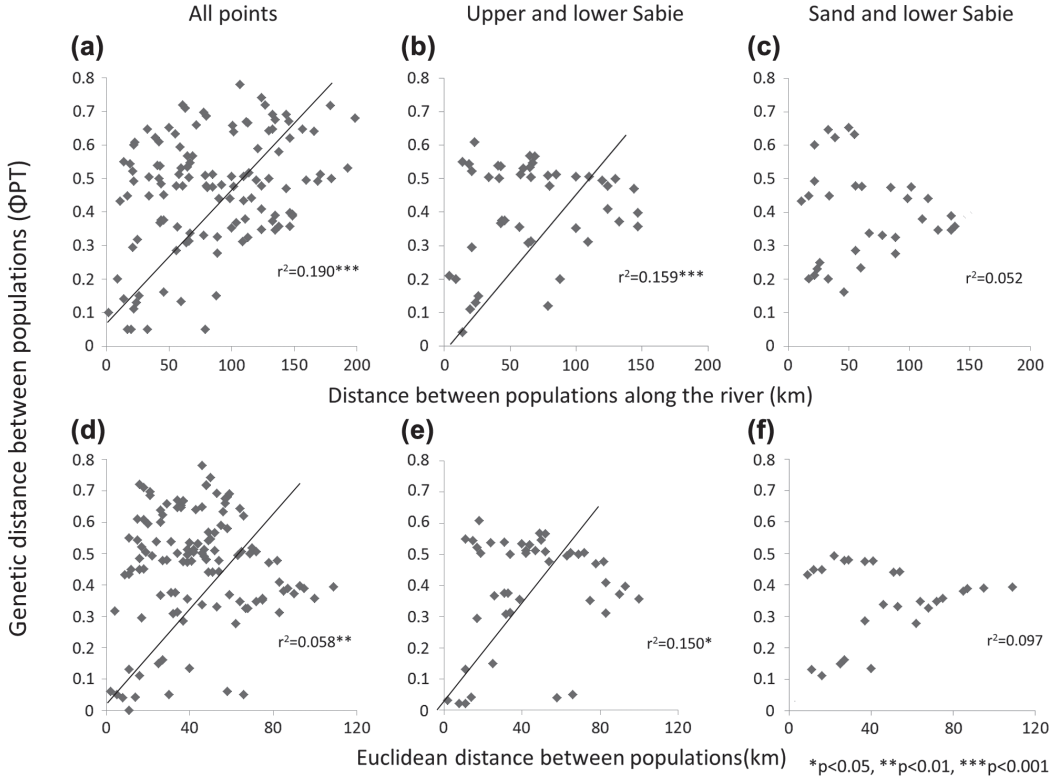


Figure 5. Relationships between pairwise genetic distance and 1) pairwise river distance and 2) pairwise Euclidean distance for selected *Lantana camara* populations, based on ISSR loci. Plot (a) and (d) is based on the full dataset, (b) and (e) on the upper and lower Sabie and, (c) and (f) on the Sand and lower Sabie.

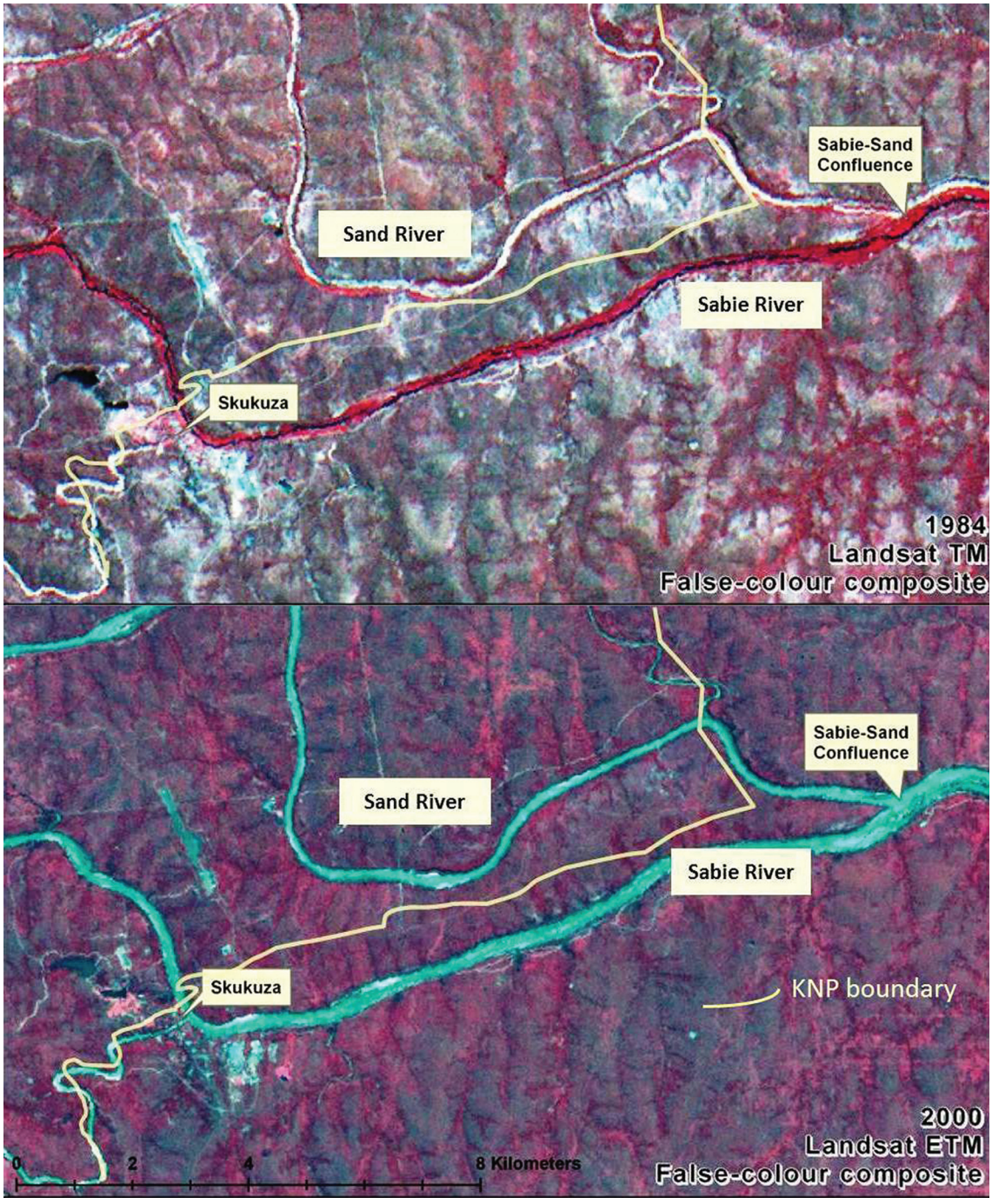


Figure 6. False-colour composite of a Landsat image, depicting a subset of the Sabie-Sand catchment in 1984 and 2000 illustrating that the 2000 flood event cleared most of the vegetation from the sides of the river. The Landsat bands were assigned as 4 – red; 3 – green and 2 – blue. As a result, vegetation within the scene appears in shades of red, with deeper reds indicating broad leaf and/or healthier vegetation while lighter reds signify grasslands or sparsely vegetated areas (Jensen 2000).

suitability for *L. camara* establishment and spread. Moreover, the north-eastern parts of South Africa experience occasional seasonal flooding during the Indian Ocean monsoonal season, and both the upper Sabie and Sand Rivers experience periodic flooding events and structural changes in vegetation in response to these events.

On the other hand, the observed genetic patterns could reflect two independent introduction events, one along the Sand and lower Sabie, and another along the upper Sabie. Therefore, what appears to be reinvasion following clearing from the Sand River may be the result of germination from existing seed banks in the lower Sabie (Vivian-Smith and Panetta 2009). To test this we reran our genetic analyses and included an 'independent' secondary source population collected from the Kwa-Zulu Natal Province, ca 500 km away from KNP. A comparison with the KNP data shows that this 'secondary source' shares no genetic relatedness to any KNP individuals (Supplementary material Appendix 4,

Fig. A2, A3). Of course, in the KNP independent source populations between different tributaries may have established long ago, diluting this type of genetic signal. However, residence time of upper Sabie populations of lantana likely far exceed those from Sand populations as this catchment is surrounded by urban areas where lantana was more likely to escape from gardens than in the Sand catchment which comprises mostly private conservation land.

There is, however, a difference in the degree to which *L. camara* populations are managed between the upper Sabie and Sand rivers. Inside the KNP management area, regular clearing efforts take place along the upper and lower Sabie, extending 100 m on each side of the rivers. In fact we could only locate three populations of *L. camara* within the lower Sabie area despite extensive search efforts along most of the river's length. Clearing along the upper Sabie has also been extended well outside the KNP to include urban areas such as Hazyview and farms (Foxcroft unpubl.). However, <20 km (one third) of the Sand tributary falls within the KNP management area, and the rest of the tributary has not been a focus of attention for KNP WfW alien plant clearing teams.

From the implications suggested by the results of this study, how can differences in management efforts influence propagule pressure to the extent that they shape population genetic structure? Management interventions between populations have previously been shown to impact the genetic structure of grassland species through local adaptation and selection (Volis et al. 2004). Likewise, flowering and seed set may be reduced in abundant populations which may in turn influence dispersal and affect genetic structure (Kleijn and Steinger 2002, Honnay and Bossuyt 2005, Peter-Schmid et al. 2008). While it has not been established, it is likely that with riparian invasive plants such effects may be more pronounced because dispersal is mostly linear and unidirectional (downstream) (Naiman and Décamps 1997).

Dispersal patterns will also likely be heavily influenced by extreme flooding events. The spread of many woody alien plant invasions are stimulated by 'normal' flood events as seed and plant parts will be washed downstream. In 2000 an extreme flooding event in KNP removed most of the vegetation (Fig. 6), including *L. camara*, from the banks of these two rivers, (Foxcroft et al. 2008b). Such floods create a template of disturbed and unoccupied habitats, with debris and other material releasing resources, which are highly suitable for (re)colonization (Davis et al. 2000). Post-flooding and continued downstream dispersal from differentially managed tributaries can thus cause significant reinvasions and would, over a short period of time, leave genetic signatures similar to those observed here for *L. camara*. Overall, it appears that *L. camara* dispersal occurs mainly downstream from both tributaries, but that due to higher propagule pressure, the Sand tributary acts as the main source for *L. camara* invasions in the lower Sabie area. To prevent continuous reinvasions of this and other landscape-riparian invaders, the Sand tributary should be incorporated into the current alien plant management plan of KNP. Since clearing efforts in and around the KNP target numerous alien taxa simultaneously, we recommend that future research should include additional invasive riparian taxa co-occurring with lantana to confirm our findings.

Our work emphasizes the importance of incorporating neighbouring areas into protected area management strategies, and understanding dispersal routes in determining which areas need to be included in defining the full domain of management efforts. Control in only one of several catchments is likely to be ineffective. It also highlights that where rivers form protected area boundaries, preventing the incursion and subsequent establishment of invasive plants is unlikely.

Furthermore, the results of this study show the ease with which *L. camara* can disperse, particularly along riparian areas, across large areas. This should be considered as part of South Africa's national management plan for the species (van Wilgen et al. 2011) so that units better suited for effective management efforts can be identified. Control efforts also need to be well documented so that an understanding of the rate of reinvasion can be considered.

This study also highlights the value of molecular techniques for determining invasion sources and pathways at landscape scales and in assessing management efficiency. Invasive plant management needs to incorporate such methods when developing control strategies to minimise reinvasions and maximize management success.

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Supplementary material (Appendix ECOG-00120 at <www.oikosoffice.lu.se/appendix>). Appendix 1–4.